Integrated CMOS Quantitative Polymerase Chain Reaction Lab-on-Chip

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Abstract

An integrated lab-on-chip capable of performing quantitative polymerase chain reaction (qPCR) is demonstrated in a high-voltage 0.35-µm CMOS process operating at a 3.3 V supply. PCR thermal cycling can be performed by physically moving droplets between three distinct temperature zones on the surface of chip or by thermal cycling a droplet in place. Droplet actuation is enabled by electrowetting-on-dielectric transport at 90 V. On-chip temperature regulation to 0.15°C is performed with on-chip resistive heaters and temperature sensors. PCR cycles are monitored by measuring the fluorescence signal of an intercalator dye using integrated single photon avalanche diodes (SPADs). Results are demonstrated for the recognition of DNA extracts from *Staphylococcus aureus* (*S. aureus*) at a detection limit of a few copies per nL target volume.

Introduction

Quantitative (or real-time) polymerase chain reaction (qPCR) is an important laboratory protocol for the amplification and subsequent quantification of a selected DNA target. With wide applicability in such areas as the diagnosis of infectious diseases and cancer and food safety, currently available qPCR techniques are among the most sensitive, able to detect just a few genetic copies in a target volume. Typical commercial qPCR instruments are table-top with typical test volumes of 10 µL with transition times between cycles of 13 to 40 seconds. While there have been many lab-on-chip (LoC) prototypes attempting to miniaturize qPCR [1], all have relied on purely passive substrates and external detectors for measurement,. The result has been a reduction of PCR cycle times to a second or less through a reduction in reaction volumes to less than 10nL but with sensitivities considerable worse than commercial machines because of the concomitant reduction in fluorescent signal [2].

In this work, we present the first use of active CMOS electronics for a qPCR LoC. The device presented in this paper performs electrowetting droplet-based transport, reagent heating, temperature sensing, and integrated fluorescence measurements on the surface of a high-voltage CMOS 0.35-µm integrated circuit. Individual droplets can be physically displaced using electrowetting-on-dielectric transport[3] among one of three temperature zones on the surface of the chip, each regulated by the heaters and temperature sensors. Alternately a droplet can be positioned in a given region and that region thermal cycled. The droplets, whose volume is less than 1nL, are able to reach thermal equilibrium in less than 500 msec. Fluorescent measurement using integrated Geiger-mode single-photon avalanche photodiodes (SPADs) allows for sensitive fluorescent detection, allowing overall sensitivities for qPCR comparable to commercial machines.

System Description

Overall architecture. The 4-mm-by-4-mm chip, shown in Fig. 1, contains a 7-by-8 array of 200-µm-by-200-µm electrodes. Rows 1 and 5 and columns 1 and 5 are fed by liquid reservoirs that can deliver droplets as small at 1 nL to the array. Droplets are aqueous in an oil medium of n-dodecane. Columns 1, 4, and 7 spanning between rows 2 and 5 constitute three temperature zones. SPAD detectors are located at the center of the 72 °C temperature region under pixel [column 7, row 3]. In normal operation, an approximately 1-nL droplet of the DNA test sample, unbonded base pairs, DNA polymerase, and intercalator dye is pulled from Droplet Reservoir 1. This droplet can be transported using the electrowetting effect over each of the three temperature zones. The fluorescence is read when the droplet completes its elongation phase. Alternately the droplet can be positioned in a single temperature zones and cycled. After thermal cycling, the droplet is transported to waste Storage Reservoir 4.

The chip is mounted on a ball-grid-array package with SU8 encapsulation of the wirebonds and chip surface except for exposing the droplet array and reservoirs. The droplet array is covered with a indium-tin-oxide-(ITO)-coated polyethylene naphthalate (PEN) coverslip to support the correct electrostatics for droplet motion.

Electrowetting operation. For droplet movement, 90 V DC signals are applied to the electrodes. This voltage is generated from an on-chip Dickson charge pump[4], as shown in Fig. 2, from a single off-chip 3.3-V supply. 3.3-V control signals are enabled at a given pixel; this control signal is level shifted to the required 90 V with the six-transistor circuit shown in Fig. 2. The use of high voltage transistors ensures that drain-bulk breakdown does not occur. Furthermore, the use of cascode transistors ML2 and MR2 in conjunction with their biasing voltage prevents the breakdown of the gate dielectrics of transistors ML3 and MR3.

Temperature control. Temperature control is maintained on the surface of the chip with on-chip sensors and heaters. Current is passed through high-resistance polysilicon resistors (Fig. 3b) to heat the chip. Resistive temperature sensors consisting of serpentine interconnect metal (Fig. 3a) are calibrated to monitor the temperature. The minimum separation of heater elements to avoid thermal smearing is shown to be 250µm (Fig. 3c). Temperature readings are accurate to 0.15 °C.

SPAD-based fluorescence measurement. In order to integrate fluorescent detection, Geiger-mode SPADs with passive quenching are integrated on-chip (Fig. 4a). The SPADs employed here have a breakdown voltage of approximately 20V. Operated at an overvoltage of 23.5V, they have a dark count rate of 100 Hz and a photon detection probability of better than 7.25% at 515 nm (Fig. 4b,c). On-chip fluorescence detection is often challenged by the need to reject the excitation source by over 50 dB in typical applications, which is not easily achieved because of the absence of adequate optical filters. In this case, we use a time-resolved measurement to achieve this background rejection. The laser excitation (at 500-nm) is pulsed and photons are detected from the fluorophores after the excitation source has been removed. Fluorescent data is collected following the elongation stage of each PCR cycle. SPAD detection limits are in the nM regime (Fig. 4d).

Measurement Results

An *S. aureus* TaqMan qPCR test kit (Norgen Biotek) is used to test chip functionality. Each 10-nL test droplet is obtained from a 50µL unit volume consisting of 10µM primer, 10µM EvaGreen Fast PCR mix, and 2ng *S. aureus* positive standard (364 basepairs long). 130 target copies exist in the 10 nL test volume. We present results for the temperature cycling of a stationary droplet according to the measured temperature profile of Fig. 5b. For comparison, 50µL samples are also processed in the MJ-Mini RT-PCR thermal cycler (Biorad Systems) at the same thermal cycling conditions. In Fig.5a, these results are normalized against the measured intensity of the qPCR chip. The 10-nL test droplets exhibit noticeable PCR amplification about 5 cycles prior than their 50uL counterparts. PCR efficiency is also noticeably greater with the qPCR chip, averaging 84.4% over the region of active amplification compared to 69.6% for the MJ-Mini results. Saturation of the curves occurs due to depletion of either the primers or unbounded base pairs.

The use of electrowetting for droplet transport allows 5000X less volume of reagent that typical commercial systems, while the integration of on-chip fluorescent detection allows this volume reduction to occur with no loss in sensitivity which has been typical of other LoC qPCR platforms. SPAD sensitivities allow for qPCR detection of a few copies per nL target volume.

References

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3. Lee, J., et al., Sensor Actuat. A. **95**, 2002. p. 259-268, 2002.
4. Chung, I. and J. Shin, IEEE JSSC, **35**, 8. p.1227-1230, 2000.

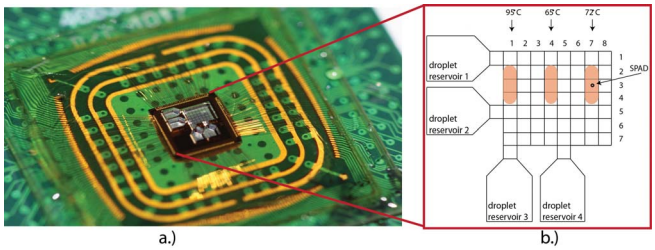

Fig. 1: PCR chip overview

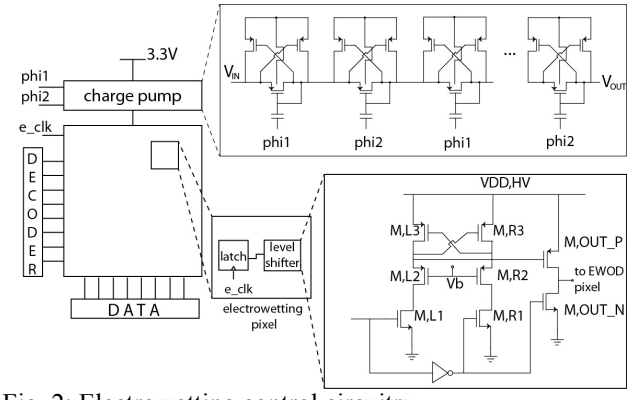

Fig. 2: Electrowetting control circuitry

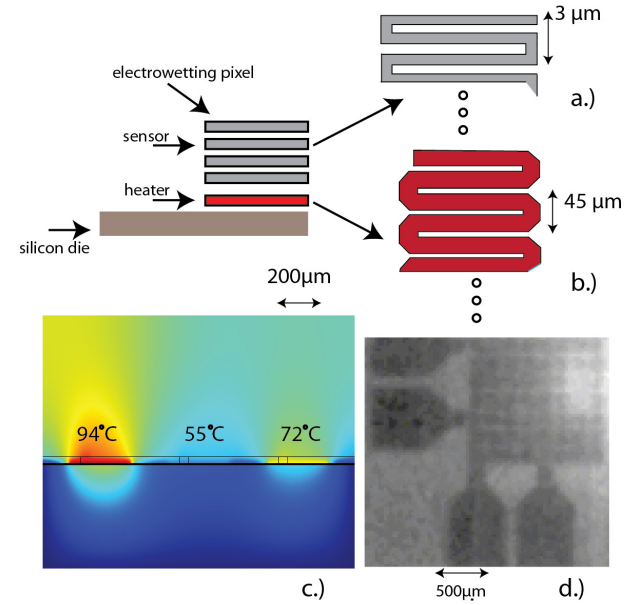

Fig. 3: On-chip thermal regulation. (a) Layout of metal-resistor temperature sensors; (b) layout of polysilicon heaters; (c) cross-sectional COMSOL simulation; (d) IR image displays distinct temperature region generated by a single polysilicon heater.

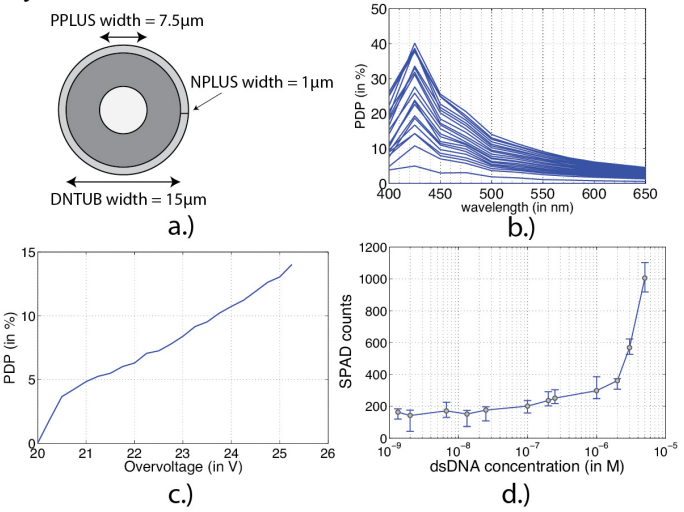

Fig. 4: SPAD characterization. (a) Device layout; (b) PDP versus wavelength; (c) PDP versus overvoltage at 515nm; (d) limit of detection obtained by varying the concentration of a 40bp test oligonucleotide in 20µM EvaGreen dye

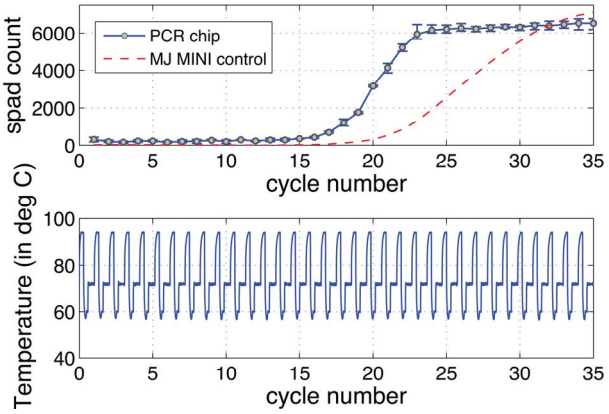

Fig. 5: PCR thermal cycling on chip. (a) Fluorescence measurements for *S. aureus* target; (b) on-chip temperature profile

Correction:

Please note that the DNA mass indicated on page 2, column 1, line 4 is incorrectly marked as 2ng. The actual mass used in the experiment is 0.25pg.